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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

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WASHINGTON, D.C. 20460

MEMORANDUM APR 3 1985

OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

SUBJECT: Glyphosate; ~~EPA~~ Reg.#: 524-308; ~~Mouse~~ oncogenicity study
Caswell #: 661A
Accession #: 251007-014

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

PC 41700

TO: Robert Taylor
Product Manager (25)
Registration Division

4/1/85

THUR: Robert P. ~~Zendzian~~, Ph.D.
Acting Head, Review Section IV
Toxicology Branch
Hazard Evaluation Division (TS-769)

FROM: William Dykstra, Ph.D. William Dykstra 3/29/85
Toxicology Branch
Hazard Evaluation Division (TS-769)

Wm W B 4/2/85

Conclusions:

1. Glyphosate was oncogenic in male mice causing renal tubule adenomas, a rare tumor, in a dose-related manner. The study is acceptable as core-minimum data.

2. The information on the oncogenicity of glyphosate was evaluated by a Toxicology Branch AD Hoc Committee which concluded that this was an oncogenic response. A copy of the consensus report of the committee is attached.

Review:

1. A chronic feeding study of Glyphosate in mice (Biodynamics # BDN-77-420; Project No. 77-2061; 7/21/83).

Test Material:

Glyphosate technical, purity = 99.7%; fine, white clumped powder; lot number, NB178260813; NB178261017.

Groups of 50 male and 50 female randomized CD-1 mice, individually caged, were administered diets containing 0, 1000, 5000, and 30,000 ppm of test material for 24 months.

Parameters evaluated were toxic signs, mortality, body weight, food consumption, water consumption and hematology at 12, 18 and 24 months.

All animals were necropsied and selected organs were weighed. Tissues were stained in H and E and examined microscopically.

Statistical analyses of the data were performed.

Results:

No treatment-related toxic signs were noted during the study. Mortality was low during the first 18 months of the study as shown in the table below as reported:

Cumulative Mortality

DOSE	Males			Females		
(ppm)	12 Mo	18 Mo	24 Mo	12 Mo	18 Mo	24 Mo
0	9	12	30	3	15	30
1,000	9	19	34	4	16	38
5,000	7	14	33	1	8	23
30,000	4	11	24	5	13	27

Body weight was consistently decreased for males and to a lesser extent, females at the 30,000 ppm dosage level during the study at several sampling intervals. Changes in body weight at the low- and mid-dose group were variable and not dose-related.

Food consumption showed no compound-related or dose-related effect. Hematological values although significant in some instances did not show a consistent dose-related response.

Necropsy did not show treatement-related lesions. There was good correlation between gross and microscopic findings. The relative and absolute weight of the testes and ovaries were increased in high dose males and females, but no histopathological finding was present as a underlying factor.

Renal tubule adenomas occurred in male mice in the following manner as reported:

Dose (ppm)	0	1,000	5,000	30,000
Number examined	49	49	50	50
Renal tubule adenoma	0	0	1	3

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They occurred in male mice 4029, 4032 and 4041 of the high-dose, and male 3023 of the mid-dose group and all were unilateral.

These tumors are rare, dose related and considered compound-related. These tumors were present at terminal kill.

Other neoplasmas were considered unrelated to treatment. No effect on latency was noted.

Significant trends and significant high-dose effects were observed in non-neoplastic lesions. The lesions considered treatment-related were hepatocyte hypertrophy, central lobular hepatocyte necrosis and chronic interstitial nephritis in high-dose males and proximal tubule epithelial basophilia and hypertrophy in high-dose females.

The table below shows the incidence of these lesions as reported:

	Control	Low	Mid	High	Linear Trend
Central lobular hepatocyte hypertrophy					
- males	9/49	5/50	3/50	17/50	b
- females	3/49	5/50	5/50	1/49	
Central lobular hepatocyte necrosis					
- males	0/49	2/50	2/50	10/50[a]	b
- females	2/49	1/50	4/49	2/49	
Chronic interstitial nephritis					
- males	5/49	2/49	7/50	12/50	b
- females	4/50	8/50	2/50	4/50	
Proximal tubule epithelial basophilia and hypertrophy					
- males	15/49	10/49	15/50	7/50	
- females	0/50	2/50	4/50	9/50[a]	a

[a]Statistically significant increase compared to control ($p \leq 0.01$) using the Chi-Square test (uncorrected for continuity).

[b]Statistically significant linear trend ($p \leq 0.01$) using the Cochran-Armitage test.

Conclusion:

Glyphosate was oncogenic in male mice producing a dose-related increased in renal tubule adenomas, a rare tumor. Dose-related non-neoplastic lesions occurred in both sexes. The NOEL for systemic effects was 5000 ppm. At the LEL, 30,000 ppm, there were increased hepatocyte hypertrophy, hepatocyte necrosis and interstitial nephritis in male mice and an increased incidence of proximal tubule epithelial basophilia and hypertrophy in female mice. Additionally, there were decreased body weights in male and female mice at 30,000 ppm which are considered compound-related.

Classification:

Core minimum data.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

MAR 4 1985

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Consensus Review of Glyphosate
Caswell No. 661A

TO: Robert Taylor
Product Manager
Herbicide - Fungicide Branch
Registration Division

On February 11, 1985, a group of Toxicology Branch personnel met to evaluate and discuss the data base on Glyphosate, and in particular the potential oncogenic response of Glyphosate.

A. The following persons were in attendance:

Theodore M. Farber, Ph.D.
Chief, Toxicology Branch

Louis Kasza, D.V.M., Ph.D.
Pathologist

Bertram Litt, Statistician

Herbert Lacayo, Ph.D.
Statistician

Reto Engler, Ph.D.

William Dykstra, Ph.D.
Reviewer

Steve Saunders, Ph.D.

Laurence Chitlik, D.A.B.T.

The signatures above indicate concurrence with this concensus report.

B. The material available for review consisted of a package issued on January 25, 1985 (attached) and a letter from Monsanto (dated February 5, 1985), rebutting the significance of renal mouse tumors.

C. Evaluation of the Facts:

1. Long-term/Pivotal Studies:

a) A 26-month rat study showed a NOEL at 30 mg/kg/day which was the HDT. The oncogenic potential at this level was negative, corroborated by an outside consultant. Although some thyroid tumors were observed in female rats in this study they were generally discounted in their significance, in and of themselves. However, it should be noted that on a mg/kg/day basis the exposure of rats was less than 1/100 of the exposure of mice (4,500 mg/kg/day). Since a toxic, or MTD, level was not reached in this study, the panel raised the conjectural issue that at toxic levels at or close to a MTD, tumors might have been induced.

b) The NOEL in a rat 3-generation reproduction study was 10 mg/kg/day. In separate teratogenicity studies feto toxic effects were noted in rats and rabbits at levels which caused significant maternal toxicity, including death; terata were not observed (ibid). These results were, however, not entered into the discussion on Glyphosate.

2. Mutagenicity Assays:

Glyphosate was tested for mutagenic activity (1) Reverse Mutation in S. typhimurium. and E. coli with and without microsomal activation, (2) Ames Assay with and without activation, (3) CHO cells with and without activation, (4) DNA repair in rat hepatocytes, (5) Rec-assay in B subtilis, and (6) Dominant lethal assay in mice. All these tests were negative, tests 1-3 are fairly well predictive of oncogenic response while 4-6 are less appropriate. An in vivo bone marrow cytogenetics study was also performed. It was negative, but scientifically not acceptable. In summary, several appropriate and scientifically acceptable tests are supportive of non-oncogenic potential of Glyphosate.

3. In the chronic mouse study carried out by Biodynamics (#BDN-77-420) renal tubule adenomas were observed in males.

Dose (ppm)	0	1000	5000	30,000
No. Exposed	49	49	50	50
Tumors	0	0	1	3

See review of W. Dykstra (dated 9/4/84).

This is a rare tumor even in Charles River CD-1 male mice. Biodynamics historical data (included in package) show that this tumor was observed only 3 times in 14 male control groups ranging in size between 51 and 60 mice.

The probability of observing this tumor 4 times or more in 198 mice (the total number of mice examined in the Glyphosate study) is p = 0.0064 when considering the historical control of the same laboratory. Even considering other reported historical controls, the p-value is low, about 0.01 indicating that it is very unlikely that the glyphosate test group is consistent with any historical controls. (See review by Dr. Lacayo).

In addition, the response rate (see above) seems to be related to the dose.

Therefore, it was the concensus of the group that the renal tubular adenomas were related to compound administration, since their frequency was not consistent with the historical controls and there is a trend indicating dose dependency.

3a. The group noted that there were other non-oncogenic, i.e., toxicological changes apparant in the kidney and liver e.g., central lobular hepatocyte hypertrophy and necrosis and chronic interstitial nephritis in males and proximal tubule epithelial basophylia and hypertrophy in females. The group discussed the possibility of kidney irritation and formulation of crystals but noted that kidney or bladder precipitaters were not reported for this assay. Therefore, a conclusion mitigating the renal tumors could not be reached. (See page 10 of contractor review).

D. Other Considerations:

The review panel recognizes that the exposure of mice was at a very high level 4.5 g/kg/day. Precipitation of Glyphosate in the kidneys might have occurred but none was reported. The panel believes that additional sectioning of new blocks of male kidneys might help in the interpretation of the study results. The kidney tumors as reported, were unilateral (pers. communication by Dr. Dykstra, after the panel meeting); additional histopathology could resolve the issue of whether this is a valid observation or due to not "finding" the tumors in the particular block analyzed.

The panel also believes that realistic exposure assessment, both for dietary and worker exposure are of singular importance. For example, the limit of detecting residue tolerances may overestimate exposure. Particular emphasis also should be given to residues in water, since Glyphosate has been used for aquatic weed control (EUP) and this use may become the subject of a permanent registration.

E. Classification of Glyphosate:

In accordance with EPA proposed guidelines (FR of Nov. 23, 1984) the panel has classified Glyphosate as a Category C oncogen.

ADDENDUM:

The letter by Monsanto (Feb. 4, 1985) has been considered in these deliberations. Several of the issues raised are, in fact, addressed in the above deliberations, although not point by point. A point by point rebuttal, including those points with little merit, will be done in addition to this evaluation.

Attachments

cc: B. Coberly
Caswell No. 661A

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

FEB 26 1985

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Use of historical data in determining the weight of evidence from kidney tumor incidence in the Glyphosate two-year feeding study; and some remarks on false positives

TO: Reto Engler, Chief
Scientific Mission Support Staff
TOX/HED/OPP (TS-769C)

FROM: Herbert Lacayo, Statistician
Scientific Mission Support Staff
TOX/HED/OPP (TS-769C)

Herbert Lacayo! Feb 26, 1985

THRU: Bertram Litt, Statistics Team Leader
Scientific Mission Support Staff
TOX/HED/OPP (TS-769C)

2/24/85

BACKGROUND

The Glyphosate feeding study (EPA Reg. #: 524-308, Caswell #: 661A, Accession #: 251007-014) on Charles River CD-1 mice generated renal tubular adenomas in male mice at the 5000 and 30000 ppm dose levels. The registrant (Monsanto) claims that such tumors are "unrelated to treatment." (ref.1). In support of that they provide historical data from Bio/dynamics and two other laboratories (ref.2).

With respect to historical data we note the large number and variety of factors which influence the life history of rodents in chronic studies. Hence, it is generally agreed that the most relevant historical controls are experiments from the subject laboratory studied within a 3 to 4 year "window" (ref.3).

SUMMARY

The main purpose of this memo is to show one way historical data may be used to evaluate the significance of tumors in the glyphosate feeding study. When these data are so used we can conclude that Glyphosate dosing has a statistically significant effect (at the p = .006 level) in the production of kidney tumors in male mice. The appropriate procedure is outlined in the next section entitled Use of Historical Data. The last Section, Remarks on False Positives, addresses some comments by Monsanto (Ref.1) on this subject. That section outlines some of the weaknesses in Monsanto's position.

USE OF HISTORICAL DATA

The following information was derived from Reference 2.

Data Source*	p (est.of tumor rate)	Sigma (est.of standard deviation)
Bio/dynamics	.00368	.00212
IRD Corp.	.00437	.00109
Combined	.00399	.00094

The value p = .00368, derived from Bio/dynamics data is a reasonable choice to use as a historical control. The data are from the same laboratory that performed the Glyphosate study and are within the appropriate 3-4 year time "window" (ref.3). Further, the standard deviation of the estimate is reasonably small.

We will now examine the Monsanto contention that the kidney tumors are unrelated to treatment. (i.e. Glyphosate has no effect on kidney tumors). First, consider the tumor rate in the Glyphosate Study: 4/198 = .0202 ---

In contrast, Bio/dynamics has the lower historical rate:

3/815 = .00368

The relevant question is: What is the probability that the 198 CD-1 mice in the Glyphosate study will produce by pure chance 4 or more mice with kidney tumors? Another way of stating this is - How likely are we to have a tumor rate of .0202 --- for the Glyphosate study given that the historical rata is .00368?

Questions of this type may be answered from manipulation of the relevant distribution which, in this case is the Binomial:

$$P(r \text{ out of } n \text{ mice have tumors}) = \binom{n}{r} p^r q^{n-r}$$

Where: n = the # of male mice in the study

r = the # of male mice with kidney tumors

p = .00368, the historical probability that an individual male mouse will develop kidney tumors.

q = 1 - p

*This does not include Hazleton Laboratories America, Inc. due to the small sample size of that data set

Using the above distribution and elementary but tedious calculations, we generate the following table:

# of mice with tumor	Probability that r or more mice will have tumors in a study with 198 male mice
r = 0	1.
1	.518177
2	.165711
3	.037443
4	.006481

This last table indicates that based on a historical rate of p= .00368 that the probability of seeing 3 or more mice with kidney tumors is about .037; and the probability of seeing 4 or more such mice (i.e. seeing what in fact happened) is about .0064. We note that even considering data from I.R.D., the p value is about .01.

Under such circumstances a prudent person would reject the Monsanto assumption that Glyphosate dosing has no effect on kidney tumor production. Another way of saying this is that if Glyphosate were truly unrelated to kidney production we would expect to see 4 or more tumors in less than 1 out of 100 experiments of the type sponsored by Monsanto. Thus, Glyphosate is suspect.

REMARKS ON FALSE POSITIVES

In ref. 1 Monsanto notes that "...if 20 types of lesions were evaluated at a probability level of .05, the number expected to be positive would not be one in 20, but rather the probability would be 64 in 100, an unacceptably high value..." Monsanto is referring to the well-known fact that by examining enough data it is likely that one will find an excess of some tumor type by chance alone; thus generating a false positive.

The Monsanto argument required the following assumptions:

1. A mouse may develop 20 distinct and independent (in the statistical sense) types of tumors.
2. The probability of each tumor type in a typical mouse is .05.

It follows from the above that:

$$P(\text{a mouse has at least one tumor}) = 1 - .95^{20} = .6415$$

Hence in 100 mice one would on the average see 64 with tumors. Monsanto proposes to avoid this "problem" of false positives by analyzing the study" ...at the .01 probability level."

We disagree with the Registrants position. First, even if one did analyze the study at the .01 level as they suggest it would still result (using the same mathematics as before) in seeing 18 mice out of 100 with tumors. And hence one still has the problem of false positives from the registrant's viewpoint. But this causes something worse from a regulatory viewpoint. We have decreased the false positive rate (i.e., the probability of saying that a chemical causes tumors when in fact it does not) at the cost of increasing the false negative rate (i.e., the probability of saying that a chemical doesn't cause tumors when in fact it does). The Registrant wishes to avoid false positives while those concerned with the public health wish to avoid false negatives. Hence, for this reason alone Monsanto's argument is unacceptable.

We further disagree as follows:

1. The two assumptions needed to support the Monsanto argument are themselves in need of support (especially the requirement for statistical independence).
2. False positive results are less likely to occur with rare tumors (ref. 5). And the tumors in question are rare.

Viewpoint is a key issue. Our viewpoint is one of protecting the public health when we see suspicious data. it is not our job to protect registrants from false positives. We sympathyze with the Registrants problem; but they will have to demonstrate that this positive result is false.

Finally, we mention that none of the tumors occurred in the control or low dose groups. Instead there was one at 5000 ppm and 3 at the 30000 ppm dose level. This together with the previous comments make it likely that there is a dose-tumor relationship for Glyphosate.

REFERENCES

1. Letter from Monsanto (signed by Frank. S. Serdy) to EPA (Attn: Robert J. Taylor) dated Feb. 5, 1985.

2. Letter from Monsanto (signed by Robert W. Street) to EPA (Attn: Robert J. Taylor) dated March 20, 1984.

3. J.K. Haseman, et al: Use of Historical Control Data in Carcinogenicity Studies in Rodents - Toxicologic Pathology - 12:126-134. 1984.

4. TOX Branch Memo from William Dykstra to Robert Taylor dated 9/4/84.

5. T.R. Fears et al: False-Positive and False-Negative Rates for Carcinogenicity. Cancer Research. 271:1941-1945. July 1977.

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